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Topical ocular pharmacokinetics and bioavailability for a cocktail of atenolol, timolol and betaxolol in rabbits

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ABSTRACT

Ocular bioavailability after eye drops administration is an important, but rarely determined, pharmacokinetic parameter. In this study, we measured the pharmacokinetics of a cocktail of three beta blockers after their topical administration into the albino rabbit eye. Samples from aqueous humour were analysed with LC-MS/MS. The pharmacokinetic parameters were estimated using compartmental and non-compartmental analyses. The ocular bioavailability was covering broad range of values: atenolol (0.07 %), timolol (1.22%, 1.51%) and betaxolol (3.82%, 4.31%). Absolute ocular bioavailability presented a positive trend with lipophilicity and the values showed approximately 60-fold range. The generated data enhances our understanding for ocular pharmacokinetics of drugs and may be utilized in pharmacokinetic model building in ophthalmic drug development.

1. Introduction

Topical administration is currently the most common route for the treatment of diseases affecting the anterior part of the eye. Ocular bioavailability after topical administration is stated to be less than 10 %, but bioavailability has been determined only for four compounds in rabbits and not at all in humans. Several pre-corneal factors, such as the drainage of excess fluid, normal tear turnover and systemic absorption through conjunctiva remove the drug from the ocular surface decreasing the drug absorption into intraocular tissues (Himmelstein et al., 1978; Lee and Robinson, 1979). Flow of drug solution into the nasolacrimal duct leads to further systemic absorption from the nasal mucosa and gastrointestinal tract (Himmelstein et al., 1978; Lee and Robinson, 1979; Urtti and Salminen, 1993).

The main ocular absorption routes after topical administration are across the cornea and conjunctiva (Fig. 1). After corneal absorption the drug permeates into the aqueous humour and further into the iris and ciliary body followed by elimination to the systemic circulation. Drug

may also be eliminated by aqueous humour turnover into the trabecular meshwork and Schlemm's canal or distribute into the lens. Transfer of drug towards the vitreous humour is hindered by the aqueous humour flow in the posterior-to-anterior direction (Maurice and Mishima, 1984). Drug may also absorb into the eye across the conjunctiva and sclera and then distribute further into the iris and ciliary body (Ahmed et al., 1987; Ahmed and Patton, 1985). Most of conjunctival drug permeation leads to systemic circulation instead of intraocular distribution.

The corneal permeation is the most important ocular absorption route for lipophilic drugs (Doane et al., 1978; Schoenwald, 1987). The corneal epithelium is the main penetration barrier in the cornea (Lach et al., 1983; Schoenwald, 1987; Schoenwald, 1990; Schoenwald and Huang, 1983). Trans-conjunctival drug absorption contributes very little to drug concentrations in the aqueous humour (Ahmed et al., 1987; Ahmed and Patton, 1985) which is the main site in the assessment of topical ocular bioavailability.

The absolute ocular bioavailability is the ratio of the dose-

Abbreviations: AUC_{inf}, area under the curve from time zero to infinity; CA, compartmental analysis; CL, clearance; C_{max}, the maximal concentration; CV%, coefficient of variation; IC, intracameral administration; ISTD, internal standards; LC-MS/MS, liquid chromatography with tandem mass spectrometry; log D_{7.4}, logarithm of the octanol-water distribution coefficient at pH 7.4; NCA, non-compartmental analysis; SE, standard error of estimates; t_{1/2}, elimination half-life; t_{max}, the time at maximal concentration; Top, topical administration; V_d, volume of distribution

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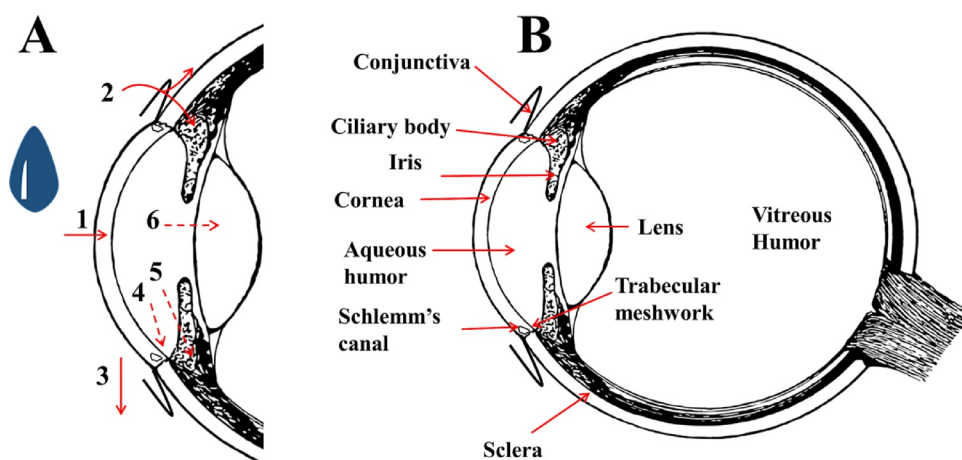


Fig. 1. Ocular pharmacokinetics after topical administration. A: Absorption and elimination pathways after topical drug administration: 1) corneal absorption; 2) conjunctival absorption; 3) clearance through nasolacrimal duct; 4) elimination to trabecular meshwork; 5) distribution to iris-ciliary body; 6) distribution to lens. B: Anatomy of the eye. (<https://www.publicdomainpictures.net/en/view-image.php?image=130389&picture=medical-eye>).

normalized areas under the concentration curve (AUC) in aqueous humour after topical and intracameral administration, respectively. In the latter case, the drug is directly injected into the anterior chamber and this situation represents 100% bioavailability.

In the present study we determined the topical pharmacokinetics for three anti-glaucoma drugs, atenolol, timolol, and betaxolol that represent a range of lipophilicity values (Fig. 2). These beta-blockers were applied topically as a cocktail on rabbit eyes, aqueous humour drug concentrations were quantified and pharmacokinetic parameters were determined including absolute ocular bioavailability (with intracameral pharmacokinetic data from our previous study (Fayyaz et al., 2019)).

2. Material and Method

2.1. Animal experiments

Animals - Sixteen male albino New Zealand rabbits, age 3–6 months and weight 2.8–3.2 kg, were used in the experiments. The animals were housed in a temperature and humidity-controlled environment with a 12/12 light/dark cycle. The animals were individually housed and fed a normal diet. All rabbits underwent an ocular examination before being accepted into experiments. Animals were handled in accordance with the statement of the Animals in Research Committee of the ARVO (Association for Research in Vision and Ophthalmology, Rockville, Maryland, USA) and all animal experiments were approved by the national Animal Experiment Board of Finland.

Topical application of the beta-blocker cocktail was performed followed by a collection of a single aqueous humour sample from each animal. The sampling times were 5, 10, 20, 30, 60, 120, 180 and

240 min, and the number of eyes at each time point were four ($n=4$). The cocktail containing 20 mM atenolol (USP reference standard, Sigma), 10 mM betaxolol hydrochloride (USP reference standard, Sigma) and 10 mM timolol maleate (USP reference standard, Sigma) in phosphate-buffered saline (DPBS, Thermofisher Scientific) (pH adjusted to 7.4; 322 mOsm/kg) was administered onto the upper cornea-scleral limbus of both eyes (25 μ L/eye) in each rabbit. The animals were sacrificed by injecting into the marginal ear vein a lethal dose of pentobarbital (Mebunat vet 60 mg/mL; Orion Pharma, Finland) and aqueous humour was aspirated from anterior chamber. All samples were cooled on ice following storage at -80°C until analysis.

2.2. Analysis of aqueous humour samples

Standards (0.1 – 5000 nM) were prepared from the beta blocker mixture in PBS and diluted with a solution containing 20% porcine aqueous humour and 80% PBS. Atenolol-d7 (Toronto Research Chemicals, Canada), betaxolol-d5 (Toronto Research Chemicals, Canada) and Rac timolol-d5 Maleate (Toronto Research Chemicals, Canada) were used as internal standards (ISTDs). The 1 mg/mL stock solutions were first prepared in DMSO and then diluted to ISTD solution containing 50 ng/mL atenolol-d7, 5 ng/mL betaxolol-d5, 5 ng/mL rac timolol-d5 maleate and 1% formic acid in acetonitrile.

Equal volumes (50 μ L) of standard solutions and ISTD solution were mixed by vortexing for 10 sec. After 15 min precipitation step the standards were centrifuged (5 min, $+4^{\circ}\text{C}$, 13000 rpm) and supernatant was collected for LC-MS analysis. Quality controls (2.5, 25, 250 and 1500 nM) in triplicates were prepared in similar manner. Aqueous humour samples were first diluted 1:5 with PBS and then ISTD solution

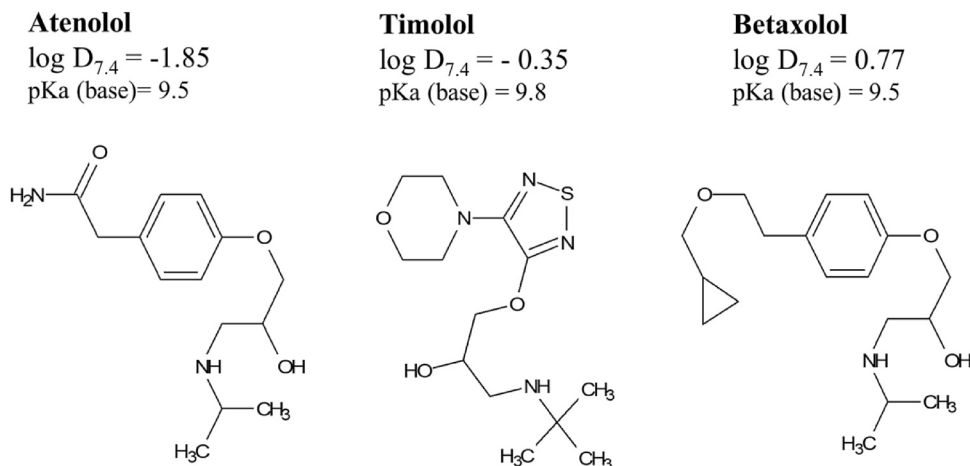


Fig. 2. The chemical structures of atenolol, timolol and betaxolol with the corresponding logarithm of the octanol-water distribution coefficient at pH 7.4 ($\log D_{7.4}$) values and pK_a values (calculated using ACD/labs, version 2020.1.1, Advanced Chemistry Development, Inc. Toronto, Canada) at pH 7.4 the relative abundance for ionized fraction versus unionized is $> 99/1$ for the three drugs.

was added. Thereafter, the procedure was similar to the handling of standards. The standards, samples and quality controls were analysed with LC-MS/MS (Agilent 1290 liquid chromatograph and Agilent 6495 triple quadrupole mass spectrometer, Agilent Technologies Inc., USA) using protocol described earlier (Fayyaz et al., 2019).

The calibration curve was prepared as duplicate and calculated as a mean of two injections using 9–11 concentration levels of total 14 levels. Calibration curves had 85–115 % mean accuracies. QC samples were 90–110% of the nominal concentrations with imprecision below 10%.

2.3. Pharmacokinetic analysis

Compartmental analysis was performed using Phoenix WinNonlin (build 8.1, Certara L.P.). Mean concentration data was analysed using one- and two-compartment models with first-order absorption kinetics. Akaike's information criterion and visual inspection of the plot of observed and predicted concentrations versus time were used to select the best compartmental model. Curve fitting was performed using three different weighting schemes: uniform, $1/\text{predicted concentration}$ ($1/Y_{\text{hat}}$) and $1/(\text{predicted concentration})^2$ ($1/Y_{\text{hat}}^2$). Coefficient of variation (CV%) of estimated parameters and residual plots were utilized for choosing the best weighting scheme within the same compartmental model. AUC from time zero to infinity ($AUC_{\text{inf,Top}}$), the maximal concentration ($C_{\text{max,Top}}$), time at the maximal concentration ($t_{\text{max,Top}}$) and elimination half-life ($t_{1/2,\text{Top}}$) were obtained following topical application of the three drugs. Non-compartmental analysis was also performed using mean concentrations and the linear trapezoidal rule (Supplementary data Table S3).

Aqueous humour bioavailability for the three beta-blockers was calculated according to Eq. 1:

$$\text{Bioavailability} = \frac{AUC_{\text{inf,Top}} \times \text{Dose}_{\text{IC}}}{AUC_{\text{inf,IC}} \times \text{Dose}_{\text{Top}}} \quad (1)$$

where Top and IC refer to topical and intracameral administration, respectively. Data for IC administration were taken from our previous study (Fayyaz et al., 2019).

3. Results

3.1. Topical pharmacokinetic parameters

The mean aqueous humour concentration data was fitted for the three beta blockers. One-compartment model was the best structural model, using the $1/Y_{\text{hat}}^2$ weighting model for all three drugs. The final model estimated concentrations are presented in Fig. 3 together with the observed data. The more lipophilic compounds betaxolol and timolol achieved higher aqueous humour concentrations than the more hydrophilic compound atenolol. Five measured aqueous humour concentrations across the three drugs were excluded from the

pharmacokinetics analysis since they were considered to be outliers (Supplementary data Table S1). The pharmacokinetic parameters estimated from the compartmental analysis are listed in Table 1.

The $AUC_{\text{inf,Top}}$ of betaxolol was 12 and 2 times higher than the dose-normalized values of atenolol and timolol, respectively and similar trends are seen for $C_{\text{max,Top}}$ (Table 1). The half-life of atenolol in the aqueous humour was 3–5 times longer than the half-lives of timolol and betaxolol.

3.2. Absolute topical bioavailabilities

The dose-normalized comparison of the concentration profiles of the three beta-blockers after topical and intracameral administration in rabbit eye (Fayyaz et al., 2019) is presented in Fig. 4.

Drug bioavailability values in aqueous humour from our topical pharmacokinetic study and the previous intracameral study (Fayyaz et al., 2019) are presented in Table 2. The order of bioavailabilities is betaxolol > timolol > atenolol based on both compartmental and non-compartmental analyses. The results show a substantial 55–62-fold difference between the bioavailability of betaxolol (3.82%, 4.31%) and atenolol (0.07%).

4. Discussion

A cocktail approach was used to determine the ocular exposure of three drugs after topical administration. This approach reduces the number of animals needed and reduces variability arising due to inter-individual differences and analytical factors. Previously, we have used the same approach to investigate the intracameral pharmacokinetics of the same drug set in the rabbit eye (Fayyaz et al., 2019). The combination of both studies allows us to determine the absolute bioavailability in aqueous humour for betaxolol, timolol and atenolol. Both compartmental analysis and non-compartmental analysis were carried out and yielded similar bioavailability values showing robust results. Ocular bioavailability is typically determined for aqueous humour, even though ciliary body is the target tissue for beta blocker anti-glaucoma drugs. The reason is that bioavailability calculation requires a direct injection into the investigated tissue for the determination of the drug clearance from the tissue. This is not feasible for iris-ciliary body, while it is possible for aqueous humour after intracameral injection.

Bioavailability is critically important parameter that provides useful information on the ocular exposure of different drugs and drugs in different formulations. Bioavailability shows the drug fraction absorbed in aqueous humour, while the concentration curves in the aqueous humour after topical administration are not only affected by absorption but also by the clearance from the aqueous humour (CL_{IC} in Table 2, Fig. 4). Unfortunately, there are only a few studies that report pharmacokinetics for both topical and intracameral administration of ophthalmic drugs (Ling and Combs, 1987; Tang-Liu et al., 1984; Yamamura et al., 1999) allowing the determination of absolute drug

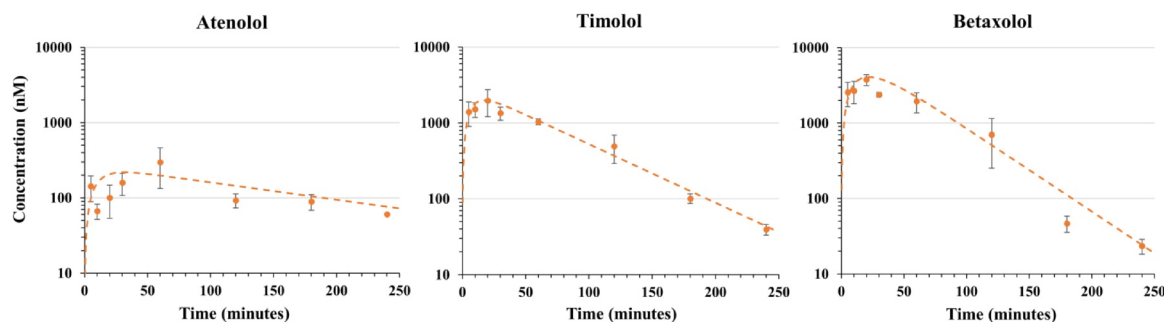


Fig. 3. Aqueous humour concentration-time profiles in rabbits after topical application of atenolol (dose = 500 nmol), timolol (dose = 250 nmol) and betaxolol (dose = 250 nmol) in a cocktail. Each circle represents the mean concentration \pm standard error of the mean ($n = 3-4$). The best fits based on one-compartmental first-order pharmacokinetic model are represented by the dashed line.

Table 1

Compartmental analysis of aqueous humour concentrations after topical administration of atenolol (dose = 500 nmol and dose-normalized values^a), timolol (dose = 250 nmol) and betaxolol (dose = 250 nmol) in rabbits. SE = standard error of the estimates.

Drug	Dose (nmol)	AUC _{inf,Top} ± SE (min*nmol/ mL)	C _{max,Top} ± SE (nmol/mL)	t _{max,Top} ± SE (min)	t _{1/2,Top} ± SE (min)
Atenolol	500	48.6 ± 15.8	0.22 ± 0.06	31.6 ± 15.5	130.4 ± 78.4
	250 ^a	24.3 ^a	0.11 ^a		
Timolol	250	152 ± 14	1.99 ± 0.20	17.3 ± 3.7	38.9 ± 2.9
Betaxolol	250	280 ± 47.8	4.07 ± 0.69	21.9 ± 5.1	27.3 ± 3.3

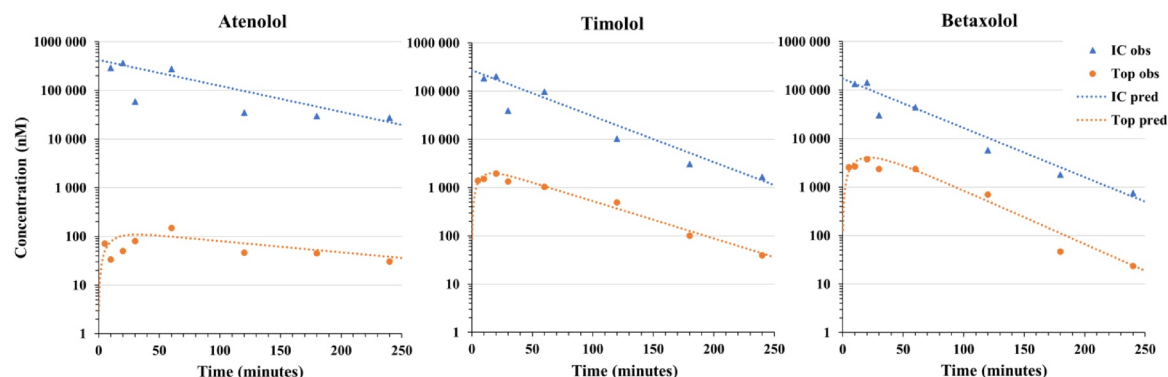


Fig. 4. Aqueous humour concentration-time profiles of atenolol, timolol and betaxolol after topical and intracameral administration. The concentrations have been normalized to the dose of 250 nmol.

Table 2

Aqueous humour bioavailability of atenolol, timolol and betaxolol using compartmental and non-compartmental analyses (CA: compartmental analysis, NCA: non-compartmental analysis).

Drugs	Dose (nmol)	Topical administration		Dose (nmol)	Intracameral administration ^a			Bioavailability	
		AUC _{inf,Top} ± SE (min*nmol/ mL)			CL _{IC} ± SE (μL/min)	Vd _{IC} ± SE (μL)	AUC _{inf,IC} ± SE (min*nmol/ mL)	(%)	
		CA	NCA				CA	CA	NCA
Atenolol	500	48.6 ± 15.8	39.2	5	6.44 ± 0.83	687 ± 140	691 ± 141	0.07	0.07
Timolol	250	152 ± 14	151	5	19.3 ± 2.66	937 ± 172	248 ± 52	1.22	1.51
Betaxolol	250	280 ± 47.8	252	5	32.2 ± 4.10	1421 ± 236	146 ± 27	3.82	4.31

Vd_{IC}: Volume of distribution after intracameral injection

CL_{IC}: Clearance after intracameral injection

^a Fayyaz et al., 2019

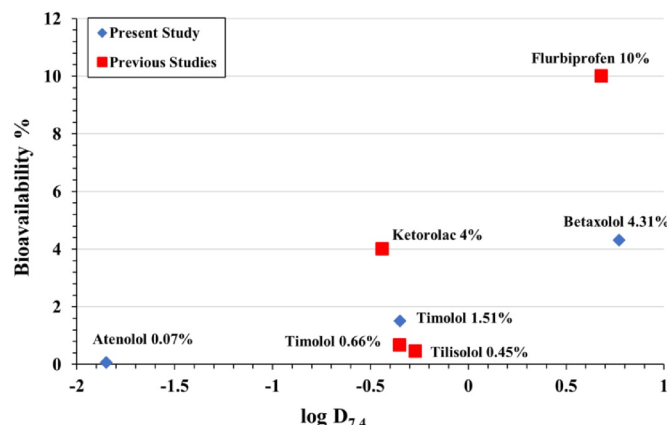


Fig. 5. Bioavailability versus log D_{7.4} of ophthalmic topical drugs in rabbit eyes of six drugs i.e. atenolol, timolol and betaxolol from present study (blue diamonds, bioavailability determined using non-compartmental analysis) and ketorolac (Ling and Combs, 1987), flurbiprofen (Tang-Liu et al., 1984), timolol and tilisolol (Yamamura et al., 1999) (bioavailability determined from non-compartmental analysis) from the literature (red squares).

bioavailability to the aqueous humour. Compiling our data with these literature studies shows a wide range of ocular bioavailabilities ranging from 0.07 to 10 % (Fig. 5). A positive trend of ocular bioavailability can be seen with lipophilicity (log D_{7.4}) as lipophilic compounds tend to have a higher ocular bioavailability than more hydrophilic compounds (Fig. 5) presumably due to the higher permeability in the cornea (Kidron et al., 2010). Plotting aqueous humour pharmacokinetic parameters (C_{max,Top}, AUC_{inf,Top}, bioavailability) against corneal permeability or cornea/conjunctiva permeability ratios in rabbit (Wang et al., 1991) results in excellent correlation (Supplementary data Figure S3 and S4). Several studies have pointed out the importance of drug lipophilicity on corneal permeability (Chien et al., 1990; Lach et al., 1983; Rusinko et al., 2007; Schoenwald, 1987; Schoenwald and Huang, 1983; Wang et al., 1991), but it is important to note that ocular bioavailability of lipophilic drugs is also limited by their fast penetration across the conjunctiva to the systemic circulation (Ramsay et al., 2018; Wang et al., 1991). Corneal penetration may also be influenced by other factors such as drug ionization, molecular size and medium (pH, osmotic pressure, other components) (Brechue and Maren, 1993; Kidron et al., 2010; Pescina et al., 2015).

Since bioavailability data for other beta-blockers are missing, we compared the dose-normalized AUC_{inf,Top} and C_{max,Top} values of drug sets in which timolol was investigated (Araie et al., 1982; Huang et al.,

1983; Ohtori et al., 1998; Ros et al., 1980; Schmitt et al., 1981; Yamamura et al., 1999). We compared the beta-blocker/timolol parameter ratios within each study (Supplementary data Table S2 and Figure S1) and observed higher $AUC_{inf,Top}$ ratio for befunolol (Araie et al., 1982), but not for tilisolol (Yamamura et al., 1999), even though both compounds are more lipophilic than timolol (Supplementary data Figure S1). Based on these pharmacokinetic parameters, we cannot conclude that higher lipophilicity necessarily results in increased drug concentrations in the aqueous humour because clearance from aqueous humour through iris-ciliary body to systemic circulation (Fig. 1) is also faster for more lipophilic compounds (Fayyaz et al., 2019). The faster clearance and shorter half-lives are also shown in Table 2 and Fig. 4. In order to quantitatively understand topical ocular pharmacokinetics, determination of bioavailability is essential.

The data variability amongst topical ocular drug studies is significant and this supports using a cocktail approach that allows generation of reliable and comparable values within the drug set. Moreover, the validity of this approach is proved when showing that one of the cocktail drugs, timolol, presents comparable pharmacokinetics to the ones reported in the literature (Supplementary data Figure S2). Even in our study some drug concentrations at 5 min were unexpectedly high compared with the later time points, especially for atenolol (Fig. 3, Supplementary data Table S1). We cannot exclude the possibility that these aqueous humour samples contained drug traces from tear fluid even though we tried to avoid the cross-contamination of the aqueous humour samples by careful sampling. In any case, these early samples have a minimal contribution to the values of $AUC_{inf,Top}$ and bioavailability, thereby they do not have any influence on our conclusions.

The present data generated for atenolol, timolol and betaxolol can be used to aid further studies with these drugs. Exact values for ocular bioavailability can be useful aid in the development of new drug delivery systems (Subrizi et al., 2019).

Conclusion

Three beta blockers were administered topically and their ocular pharmacokinetics were evaluated. Absolute bioavailability of atenolol, timolol and betaxolol was quantitated in aqueous humour. The data shows broad, about 60-fold, range of bioavailability for topical beta blocking agents. The outcomes of this study is for improved understanding on ocular pharmacokinetics and may inform ophthalmic topical drug dosing and drug development.

CRediT authorship contribution statement

Anam Fayyaz: Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing. **Veli-Pekka Ranta:** Methodology, Writing - review & editing. **Elisa Toropainen:** Methodology, Investigation. **Kati-Sisko Vellonen:** Methodology, Investigation. **Annika Valtari:** Methodology, Investigation. **Jooseppi Puranen:** Methodology, Investigation. **Marika Ruponen:** Project administration, Investigation, Writing - review & editing. **Iain Gardner:** Supervision, Writing - review & editing. **Arto Urtti:** Conceptualization, Supervision, Writing - review & editing, Resources. **Masoud Jamei:** Conceptualization, Supervision, Writing - review & editing, Resources. **Eva M. del Amo:** Conceptualization, Validation, Supervision, Project administration, Writing - review & editing.

Declaration of Competing Interest

Anam Fayyaz, Masoud Jamei, and Iain Gardner are employees of Certara UK Limited, Simcyp Division.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.ejps.2020.105553](https://doi.org/10.1016/j.ejps.2020.105553).

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